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# Haemoglobin adducts from aromatic amines in children in relation to area of residence and exposure to environmental tobacco smoke

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**Abstract** ***Objective***: The influence of area of residence on haemoglobin (Hb) adducts of 4-aminobiphenyl (4-ABP), *o*-, *m*-, *p*-toluidine and *o*-anisidine was investigated in children from three different-sized Bavarian cities – Munich, Augsburg and Eichstätt, with 1,300,000, 250,000 and 13,000 inhabitants, respectively – and was compared with that of exposure to environmental tobacco smoke (ETS). ***Methods***: Blood samples from Munich ($n = 34$) and Eichstätt ($n = 64$) were from children attending the Paediatric Clinic of the Technical University of Munich (TUM) or a practice in Eichstätt, respectively. Blood samples ($n = 126$) together with urine samples ($n = 88$) were collected from Augsburg children during school medical examination. Personal data including possible sources of ETS exposure were obtained at the interview. Hb adduct levels were analysed by a gas chromatographic method, using mass spectrometry with selected-ion monitoring. Urinary cotinine was determined by radioimmunoassay. ***Results***: 4-ABP Hb adduct levels in children from Munich were 1.5 and 1.2 times higher than those in children from Eichstätt and Augsburg ($P < 0.001$). Children from Munich also had significantly higher Hb adduct levels of monocyclic aromatic amines than did children from Eichstätt and, except for *o*-toluidine, children from Augsburg ($P < 0.005$). Compared with children from Eichstätt, children from Augsburg had higher Hb adduct levels of 4-ABP, *o*- and *m*-toluidine ($P < 0.01$) but not *p*-toluidine and *o*-anisidine. In a multivariate analysis, gender, age and body mass index had no consistent influence on Hb adducts. ETS exposure resulted in a slight, nonsignificant increase in 4-ABP Hb adduct levels. In contrast, adduct levels from monocyclic aromatic amines were consistently decreased in ETS-exposed children (significant for *o*- and *m*-toluidine, $P < 0.05$). ***Conclusions***: Hb adducts from aromatic amines in children were strongly influenced by site of residence, whereas ETS exposure did not significantly increase the adduct levels.

**Key words** Haemoglobin adducts · Aromatic amines · Children · Regional difference · Passive smoking

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## Introduction

A weak association between exposure to environmental tobacco smoke (ETS) and lung cancer has been repeatedly found in epidemiological studies (Dockery and Trichopoulos 1997). In a meta-analysis of 30 studies the relative risk for lung cancer in nonsmoking women exposed to ETS was estimated to be 1.19 (90% confidence interval 1.04–1.35; US EPA 1992). However, because of

potential confounding the validity of relative risks well below 2 is questionable (Taubes 1995; Überla 1998). A recent multicentre case-control study in Europe, one of the largest and most exhaustive examinations, failed to show a statistically significant increase in risk by spousal and/or workplace exposure to ETS (combined odds ratio for ever-exposure = 1.14: 95% confidence interval = 0.88–1.47). In contrast, ETS exposure during childhood resulted in a significantly decreased risk of lung cancer (odds ratio for ever-exposure = 0.78: 95% confidence interval = 0.64–0.96; Boffetta et al. 1998). In order to improve the weak evidence of an ETS-related risk, biomarker studies on dose and effect of ETS exposure were performed (Scherer and Richter 1997). A significant, higher uptake of carcinogens from ETS than from other environmental sources would substantially contribute to the plausibility of a true increase in cancer risk by ETS (Blot and McLaughlin 1998). One of the most prominent examples of such an increased exposure is the study by Hammond et al. (1993) showing a significant correlation between Hb adduct levels from the human carcinogen 4-aminobiphenyl (4-ABP) and exposure to ETS in nonsmoking pregnant women. However, in a more recent study we were not able to confirm this finding. Hb adducts from neither 4-ABP nor from other aromatic amines or from tobacco-specific nitrosamines showed a relationship to ETS exposure in pregnant women (Branner et al. 1998). Therefore, in our view the major source of these adducts in nonsmokers is still unknown. In previous studies, we found evidence that aromatic amine Hb adduct levels are lower in people living in rural areas compared with urban environments (Falter et al. 1994).

The objective of this study was to elucidate the influence of place of living and ETS exposure on Hb adduct levels of aromatic amines in nonsmokers. For this purpose Hb adduct levels were measured in children living in three cities which largely differ in their sizes.

## Subjects and methods

### Subjects

The study was performed with children living in three cities in Southern Germany: Munich, the capital of Bavaria with 1.3 million inhabitants; Augsburg, 30 miles west of Munich, with 250,000 inhabitants; Eichstätt, 45 miles north of Munich, with 13,000 inhabitants. Only the erythrocyte fractions of blood samples drawn for other purposes were used in the present study. Written consent from the children's parents or guardians was obtained for each sample.

In Spring 1996, all 2,444 6 to 7-year-old children from Augsburg starting school in fall 1996 were invited to take part in an allergological and dermatological investigation, called MIRIAM (Multicentric International Study for Risk Assessment of Indoor and Outdoor Air on Allergy and Neurodermitis Morbidity). The purpose of the MIRIAM study was to evaluate the impact of outdoor and indoor air pollution on sensitizations and allergies in children. The investigation was combined with the school entrance medical examination which is compulsory for all school beginners, and 1,669 (69%) children agreed to participate. During the first 6 weeks of the study, the erythrocyte fractions of blood samples from 126 randomly selected children were obtained for determination of aromatic amine Hb adducts. Spot urine samples were available from 88 of the 126 children. Blood samples from the Munich subjects were obtained during fall 1996 from 34 children attending the Paediatric Clinic of the Technical University of Munich. All children were diagnosed for general clinical symptoms and for atopic allergy according to the criteria of the ongoing GINI (German Infant Nutritional Intervention) programme. Briefly, the diagnosis of atopic allergy was based on clinical symptoms, positive skin "prick" test and the negative radio allergen sorbent test (RAST). For Eichstätt, blood samples were provided by the paediatrician Dr. K. Wenk from 65 children consulting the practice for routine check-ups or various diseases during fall 1996.

For all children from Munich and Eichstätt a single page questionnaire was completed by the investigators, addressing questions of general health, life-style, and exposure to ETS. From the Augsburg children a validated questionnaire with 70 questions on air-way diseases, allergies and potentially confounding factors was obtained. The sociodemographic data are summarized in Table 1. Based on the subjects' living conditions, exposure to ETS was stratified into three categories: A, no ETS exposure; B, exposure by household members other than the mother; C, exposure via maternal smoking.

A blood sample (10 ml) was collected in EDTA-treated Vacutainers. A spot urine sample was obtained from 88 of the 126 children from Augsburg and stored at –20 °C. Plasma was separated from blood cells by centrifugation and the blood cells were washed three times with 8 ml of saline. Blood samples from Augsburg and Eichstätt were processed immediately and stored at –20 °C prior to shipment to our laboratory on dry ice. Samples from Munich were kept at 4 °C and transported on ice within 6 h to our laboratory for further processing.

### Analytical methods

*Hb adducts*

Aromatic amine Hb adducts were determined as previously described, with some minor modifications (Kutzer et al. 1997). Briefly, Hb solutions obtained after centrifugation of lysed red

**Table 1** Sociodemographic data of the study population. ETS score gives numbers of children with *A* no ETS exposure, *B* exposure by household members other than the mother, *C* exposure to maternal smoking; mean ± SD (*n*; min./max.)

| Characteristic | Munich | Augsburg | Eichstätt |
|---|---|---|---|
| Total number | 34 | 126 | 64 |
| Size of city | 1,300,000 | 250,000 | 13,000 |
| Gender (male/female) | 25/9 | 64/59 | 36/28 |
| Age (years) | 9.3 ± 2.3 (34; 5/15) | 6.3 ± 0.3[b] (118; 5.9/6.9) | 8.2 ± 3.6[c] (63; 0.25/14) |
| BMI (kg/m) | 17.3 ± 2.9 (27; 11.2/23.1) | 16.0 ± 1.9[a] (118; 10.8/22.7) | 16.7 ± 2.7 (63; 12.3/23.3) |
| ETS exposure (h/d) | 3.28 ± 1.94 (17; 0.25/8.0) | 6.50 ± 6.41 (46; 1.0/24.0) | 1.95 ± 0.98 (16; 0.25/3.5) |
| ETS score | | | |
| A | 15 (45%) | 65 (52%) | 39 (61%) |
| B | 5 (15%) | 31 (25%) | 16 (25%) |
| C | 13 (39%) | 27 (22%) | 9 (14%) |

[a] Significantly different from Munich; $P < 0.05$
[b] Significantly different from Munich; $P < 0.0001$
[c] Significantly different from Augsburg; $P < 0.001$

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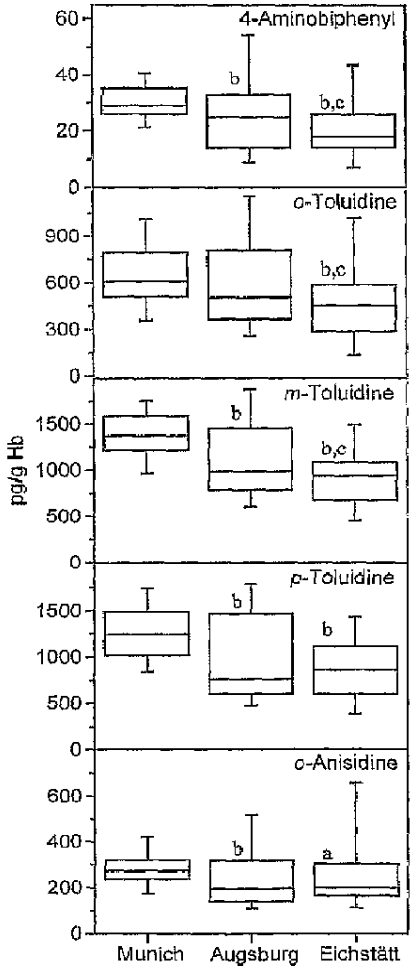


**Fig. 1** Regional differences in aromatic amine Hb adduct levels. *Boxes* cover 25th and 75th percentiles with median levels indicated as *horizontal lines*. Outer horizontal lines indicate 5th and 95th percentiles. [a]Significantly different from Munich, $P < 0.01$, [b] significantly different from Munich, $P < 0.001$, [c]significantly different from Augsburg, $P < 0.01$

blood cells were dialysed against 20-times the volume of deionized water for 2 days with three changes of the water. Hb content was determined by Drabkin's assay (Sigma, Deisenhofen, Germany). The samples were divided into two equal parts. After mild base-catalysed hydrolysis 78 pg $D_4$-*p*-toluidine (a gift from Prof. Sabbioni, Walther Straub Institute, Munich, Germany) and 81 pg $D_9$-4-ABP (IC Chemikalien, Munich, Germany) were added as internal standards. Extraction, clean-up and concentration were performed by a one-step procedure using $C_{18}$ cartridges (Varian, Darmstadt, Germany). The cartridges were eluted in three steps with 1.5, 1.0 and 0.75 ml $CHCl_3$, and the combined $CHCl_3$ extracts were concentrated in a vacuum centrifuge. The aromatic amines were derivatized with pentafluoropropionic anhydride and analysed by capillary gas chromatography-mass spectrometry with negative chemical ionization and selected-ion monitoring. The analytical limit of detection was 0.5–1 pg adduct/g Hb using a 5-ml aliquot of blood. All samples were analysed in duplicate. Two blank water samples were analysed each day to control for background contamination.

*Urinary cotinine*

Cotinine in urine was determined by a radioimmunoassay according to the method of Langone et al. (1973), with modifications by Haley et al. (1983). The limit of detection was 1 ng/ml. Creatinine in urine was determined by the Jaffé method using a commercial test kit (Merck, Darmstadt, Germany). Results on cotinine are given as the cotinine (μg) to creatinine (g) ratio (CCR).

Statistical analysis

When not stated otherwise, results are given as arithmetic means and standard deviations (SDs). All haemoglobin adduct concentrations were normally distributed after logarithmic transformation (base 10). Therefore, all further statistical tests were done after this transformation. Comparison of group means was performed by Student's *t*-test. The influence of ETS adjusted for place and sociodemographic parameters, as well as the influence of place adjusted for ETS and sociodemographic parameters, was determined by linear regression. The resulting parameter estimates (b) were transformed: $f(b) = 10^b = a$, where a can be interpreted as an adjusted quotient of geometric means (mean ratio, MR), indicating the change in geometric means when the independent variable changes by one unit. For binary variables (ETS yes or no, for instance) MR is the adjusted change in geometric means when the factor is present, compared with the case when the factor is not present. If the respective 95% confidence interval (95% CI) does not include 1 (= no change) the result is judged significant.

## Results

Hb adducts of 4-ABP, *o*-, *m*-, *p*-toluidine, and *o*-anisidine were detectable in all blood samples. Duplicate analyses revealed a mean coefficient of variation of 4%. In all but one sample the adduct levels of 3-aminobiphenyl were below the limit of detection (1 pg/g Hb). In children from Eichstätt, four Hb adduct levels each of *m*-toluidine and *p*-toluidine (< 176 and < 79 pg/g Hb, respectively) were below the threefold interquartile difference from the median values and were, therefore, regarded as outliers, and were eliminated from the statistical analyses.

All aromatic amine Hb adduct levels were highest in children from Munich, intermediate in children from Augsburg and lowest in children from Eichstätt (Fig. 1, Table 2). The differences in biomarkers between children from Munich and Eichstätt were statistically significant for 4-ABP (1.5-fold; $P < 0.001$), *o*- and *p*-toluidine (1.3 to 1.6-fold; $P < 0.001$) as well as *o*-anisidine (1.1-fold; $P = 0.004$). With the exception of *o*-toluidine (1.1-fold; $P = 0.058$) adduct levels were also statistically higher in children from Munich than in children from Augsburg (1.2–1.3 times; $P < 0.001$). The Hb adduct levels in children from Augsburg were significantly higher than in children from Eichstätt for 4-ABP (1.3 times; $P = 0.009$), *o*-toluidine (1.2 times; $P = 0.003$) and *m*-toluidine (1.3 times; $P = 0.002$) but not *p*-toluidine and *o*-anisidine.

With only two exceptions, all differences between children from Eichstätt and Munich were nearly identical and remained significant when children from non-smoking (ETS no) and smoking homes (ETS yes) were

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Table 2 Regional differences in haemoglobin adducts of aromatic amines (pg $g^{-1}$ Hb) in children; means ± SD ($n$) Statistical differences (by Student's $t$-test; ) are determined between towns in corresponding groups (all, ETS yes, ETS no) and within a town between ETS yes or no

| Hb adduct | | Munich | Augsburg | Eichstätt |
|---|---|---|---|---|
| 4-ABP | All | 30.7 ± 6.1 (33) | 26.6 ± 17.5 (123)[b] | 20.7 ± 11.8 (64)[b,c] |
| | ETS no | 30.1 ± 7.0 (15) | 25.0 ± 14.8 (65)[b] | 19.0 ± 7.9 (39)[b] |
| | ETS yes | 31.2 ± 5.5 (18) | 28.5 ± 20.0 (58)[a] | 23.4 ± 15.9 (25)[a] |
| *o*-Toluidine | All | 632 ± 206 (33) | 598 ± 298 (123) | 487 ± 295 (64)[b,c] |
| | ETS no | 620 ± 206 (15) | 621 ± 327 (65) | 558 ± 328 (39)[f] |
| | ETS yes | 642 ± 212 (18) | 574 ± 262 (58) | 376 ± 191 (25)[b,d] |
| *m*-Toluidine | All | 1,384 ± 259 (33) | 1,115 ± 416 (123)[b] | 935 ± 408 (60)[b,c] |
| | ETS no | 1,410 ± 287 (15) | 1,143 ± 418 (65)[b] | 1,014 ± 439 (37)[b,e] |
| | ETS yes | 1,363 ± 240 (18) | 1,084 ± 422 (58)[b] | 809 ± 321 (23)[b,c] |
| *p*-Toluidine | All | 1,254 ± 279 (33) | 991 ± 485 (123)[b] | 866 ± 408 (60)[b] |
| | ETS no | 1,229 ± 258 (15) | 1,018 ± 498 (65)[b] | 906 ± 367 (38)[b] |
| | ETS yes | 1,275 ± 300 (18) | 944 ± 468 (58)[b] | 795 ± 302 (22)[b] |
| *o*-Anisidine | All | 284 ± 73 (33) | 242 ± 129 (123)[b] | 254 ± 179 (64)[a] |
| | ETS no | 275 ± 81 (15) | 256 ± 144 (65) | 274 ± 200 (39) |
| | ETS yes | 292 ± 66 (18) | 225 ± 111 (58)[b] | 222 ± 138 (25)[a] |

[a] Significantly different from Munich; $P < 0.01$
[b] Significantly different from Munich; $P < 0.001$
[c] Significantly different from Augsburg; $P < 0.01$
[d] Significantly different from Augsburg; $P < 0.001$
[e] Significantly different from children exposed to ETS; $P < 0.05$
[f] Significantly different from children exposed to ETS; $P < 0.01$

compared separately (Table 2). For *o*-toluidine and *o*-anisidine the differences in children from nonsmoking households were weaker and not more significant. Similar results were obtained for the differences between children from Augsburg and Munich, which remained significant after separate analysis according to the ETS exposure with the exception of *o*-anisidine. For the comparison of children from Augsburg and Eichstätt, the significant differences for *m*-toluidine remained, whereas the differences for 4-ABP were lost in both subgroups and for *o*-toluidine and *o*-anisidine only in children from nonsmoking homes.

In all three towns children from nonsmoking homes had nonsignificantly lower 4-ABP adduct concentrations than children from smoking homes (Table 2). In contrast, monocyclic aromatic amine adduct levels were always higher in children from nonsmoking homes in Augsburg and Eichstätt, reaching significance for *o*- and *m*-toluidine in children from Eichstätt (1.8- and 1.3-fold, $P < 0.01$). In children from Munich no influence on monocyclic aromatic amine adduct levels by smoking status of the households was obvious.

The validity of self-reported ETS exposure was confirmed by determination of urinary cotinine in children from Augsburg. Children from smoking homes ($n = 38$, 48.1 ± 40.7 μg cotinine/g creatinine) had a more than 3-times higher CCR than children from nonsmoking homes ($n = 50$, 13.9 ± 12.7 μg/g, $P < 0.001$). In contrast, Hb adduct levels were not significantly different between exposed and nonexposed children. Whereas 4-ABP adducts were slightly higher in children from smoking than in children from nonsmoking homes (29.3 ± 21.7 versus 23.3 ± 14.4 pg/g, $P = 0.058$), adducts from monocyclic amines were consistently decreased by about 10%–20% (differences not significant, $P > 0.1$). As shown in Fig. 2, children exposed to ETS via maternal smoking ($n = 19$) had a significantly higher CCR (62.4 ± 49.5 μg/g) than children exposed to ETS by household members other than the mother ($n = 19$, 33.8 ± 23.1 μg/g, $P = 0.031$), and in both exposed groups the CCR was significantly higher than in children from nonsmoking households ($P < 0.001$). In contrast, 4-ABP Hb adduct levels were not increased in children with smoking mothers compared with children exposed to ETS by other household members (29.1 ± 24.0 versus 29.6 ± 19.9 pg/g).

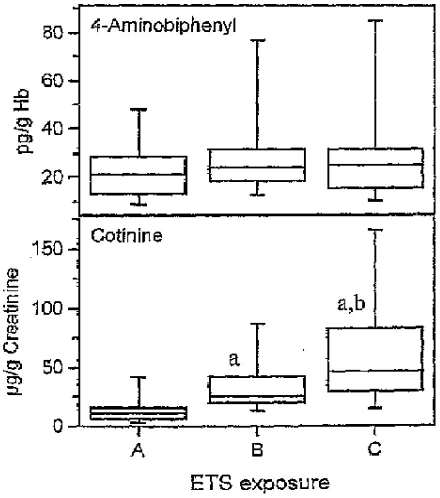


Fig. 2 Influence of ETS exposure on 4-ABP Hb adduct levels and urinary cotinine excretion in 88 children from Augsburg. ETS exposure groups: *A* no ETS exposure, *B* exposure by household members other than the mother, *C* exposure to maternal smoking. *Boxes* cover 25th and 75th percentiles, with median levels indicated by *horizontal lines*. Outer *horizontal lines* indicate 5th and 95th percentiles. [a]A < B and A < C, $P < 0.001$; [b]B < C, $P = 0.031$

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**Table 3** Result of linear regression; adjusted mean ratios (*MR*) and 95% confidence intervals (*95% CI*) with *P* values

| Characteristic | 4-ABP ($n$ = 207) | | | $o$-Toluidine ($n$ = 207) | | | $m$-Toluidine ($n$ = 204) | | | $p$-Toluidine ($n$ = 203) | | | $o$-Anisidine ($n$ = 207) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | MR | 95% CI | $P$ < | MR | 95% CI | $P$ < | MR | 95% CI | $P$ < | MR | 95% CI | $P$ < | MR | 95%CI | $P$ < |
| Gender | | | | | | | | | | | | | | | |
| Female | 1.00 | | | 1.00 | | | 1.00 | | | 1.00 | | | 1.00 | | |
| Male | 0.99 | 0.86–1.15 | | 0.94 | 0.82–1.08 | | 0.91 | 0.82–1.01 | 0.10 | 0.88 | 0.78–0.99 | 0.05 | 1.03 | 0.90–1.18 | |
| Age (per year) | 0.97 | 0.94–1.01 | | 1.00 | 0.96–1.04 | | 0.97 | 0.94–1.00 | 0.05 | 0.98 | 0.95–1.01 | | 1.01 | 0.97–104 | |
| BMI (per unit) | 1.02 | 0.99–1.06 | | 1.01 | 0.98–1.05 | | 0.99 | 0.97–1.02 | | 0.98 | 0.95–1.01 | | 0.98 | 0.95–1.01 | |
| ETS | | | | | | | | | | | | | | | |
| No exposure | 1.00 | | | 1.00 | | | 1.00 | | | 1.00 | | | 1.00 | | |
| Smoking home | 1.10 | 0.95–1.27 | | 0.86 | 0.74–0.98 | 0.05 | 0.88 | 0.80–0.98 | 0.05 | 0.90 | 0.79–1.01 | 0.01 | 0.91 | 0.79–1.04 | |
| City | | | | | | | | | | | | | | | |
| Eichstätt | 1.00 | | | 1.00 | | | 1.00 | | | 1.00 | | | 1.00 | | |
| Augsburg | 1.18 | 0.99–1.40 | 0.10 | 1.27 | 1.08–1.50 | 0.01 | 1.14 | 1.00–1.29 | 0.05 | 1.01 | 0.87–1.17 | | 0.99 | 0.85–1.17 | |
| Munich | 1.68 | 1.32–2.16 | 0.01 | 1.40 | 1.11–1.77 | 0.01 | 1.68 | 1.41–1.99 | 0.01 | 1.55 | 1.26–1.92 | 0.01 | 1.26 | 1.02–1.59 | 0.05 |

The results of linear regression analysis on the influence of gender, age, body mass index (BMI), ETS exposure and place of residence on Hb adduct levels are given in Table 3. Whereas BMI did not show any significant effect, the levels of Hb adducts of the toluidine isomers tended to be lower in boys than in girls. Age was without effect on Hb adducts except for a slight decrease in $m$-toluidine adduct levels with increasing age. Children living in smoking homes exhibited a small, nonsignificant increase in 4-ABP adduct levels and a small but significant decrease in adduct levels from toluidines. The linear regression analysis confirmed the regional differences in Hb adducts from aromatic amines. Using children from Eichstätt as a reference group, we found that MRs were consistently greater than 1.0, reaching significance for all aromatic amines in children from Munich and for $o$-toluidine and $m$-toluidine in children from Augsburg.

## Discussion

In this study internal exposure to aromatic amines has been studied in children by an established analytical method (Tannenbaum and Skipper 1994) slightly modified by us (Kutzer et al. 1997), and acknowledged after further minor modifications by the German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area (Lewalter and Gries (2000). Adducts from 4-ABP, $o$-, $m$- and $p$-toluidine isomers, as well as from $o$-anisidine, were detected in all samples. With the exception of $m$-toluidine, all other amines have been classified as carcinogens (Sabbioni and Richter 1999). The adduct levels of 4-ABP were in the same concentration range as the levels reported in children from New York (Tang et al. 1999) and in adult nonsmokers (Bartsch et al. 1990; Bryant et al. 1987; Falter et al. 1994; Hammond et al. 1993, Pinorini-Godly and Myers 1996; Riffelmann et al. 1995). Adducts from monocyclic aromatic amines have been determined for the first time in children. The concentrations of toluidines are 2–3 times higher than reported for adults (Bryant et al. 1988; Falter et al. 1994). A possible explanation for this discrepancy could be that we used a more appropriate internal standard, $D_4$-$p$-toluidine, compared with $D_5$-aniline in the earlier studies. Using this internal standard we realized that in our original analytical method (Kutzer et al. 1997) the elution of the toluidines from the $C_{18}$ cartridges was incomplete. A better recovery together with a more precise calculation based on the new internal standard could have led to the higher values in this study. The presence of $o$-anisidine Hb adducts in children is not surprising. Adducts from this amine have been detected previously in adults from Germany (Branner et al. 1998, Falter et al. 1994). The origin of this adduct is unknown. o-Nitroanisole, a possible precursor, was released into the environment in the course of an accident in a German chemical plant (Hauthal 1993).

Our findings show that children living in Eichstätt, a small town with a largely rural environment, had considerably lower aromatic amine Hb adduct levels than children from larger cities such as Augsburg and Munich. Tobacco smoke is certainly not the only source, and probably not the most important one, because there was no significant increase in these adducts with exposure of the children to ETS (Tables 2 and 3). At present, little is known about sources other than tobacco smoke for the human uptake of aromatic amines or their corresponding nitro-compounds, e.g. 4-nitrobiphenyl, as precursors (Bryant et al. 1987). Air pollution associated with traffic density could be a source of aromatic amine Hb adducts which may arise from the uptake of nitroaromatics present in diesel exhaust (Bryant et al. 1987; Ries 1992). Kerosene heaters, e.g. open-type oil-burning heaters (Tokiwa and Ohnishi 1986), were used in only a few households of our study subjects and were not associated with increased Hb adduct levels. Other possible sources for these amines or their nitroaromatic precursors, including food (Bryant et al. 1987; Neurath et al. 1977; Richter et al. 2000; Vitzthum et al. 1975) and drinking water (Djozan and Faraj-Zadeh 1995; Fattore et al. 1998; Müller et al. 1997; Neurath et al. 1977) or azo-dyes (Bryant et al. 1987; Oh et al. 1997; Platzek et al. 1999) are not expected to differ significantly between the three cities.

Selection bias is not likely to explain these results. Age and BMI had no influence on Hb adduct levels. The tendency for lower adduct levels in boys compared with girls would have rather diminished the differences between Munich (74% boys) and the other cities (52% boys in Augsburg and 56% boys in Eichstätt). To the best of our knowledge, these variables have not been addressed in other studies on Hb adducts from aromatic amines.

In a smaller study with 51 preschool children from New York, Tang et al. (1999) measured 4-ABP Hb adduct levels of 32 ± 2 pg/g Hb, similar to our values for children from Munich (31 ± 6 pg/g). In a subgroup of ten children living in nonsmoking households in New York the adduct levels were 31% lower than in 41 New York children living in smoking homes (Tang et al. 1999). This difference is higher than the difference of 10% which we observed after adjustment in our 207 children. However, the difference in the New York children, 47 Hispanic and four African-American subjects, reached significance ($P < 0.05$) only after adjustment for ethnicity. Ethnicity did not play a role in our study since all children were of Caucasian origin. One reason for the higher impact of ETS exposure on 4-ABP Hb adduct levels in the study from Tang et al. (1999) compared with our study could be the much higher extent of ETS exposure in the New York children. Plasma cotinine levels in children living in smoker households were 2.87 ± 5.61 ng/ml, compared with 0.264 ± 0.596 ng/ml in children from nonsmoking households, an 11-fold difference. In our study, the Augsburg children from smoking homes had only 3.5-times higher urinary cotinine levels than children from nonsmoking homes. However, when we compared the 23 children with the highest CCR with the 23 children with the lowest CCR, thereby obtaining a similar 11-fold difference in ETS exposure (65.0 ± 8.1 versus 6.1 ± 0.5 μg/g; $P < 0.001$) compared with the study from Tang et al. (1999), no difference in 4-ABP Hb adduct levels (23.9 ± 1.9 versus 24.3 ± 3.5 pg/g; $P > 0.1$) was seen. Interestingly, in this subgroup, Hb adduct levels of all three toluidine isomers were significantly lower in the highly exposed children from smoking homes than in the children from nonsmoking homes (*o*-toluidine: 574 ± 58 versus 757 ± 66 pg/g, $P = 0.042$; *m*-toluidine: 1,119 ± 83 versus 1,384 ± 87 pg/g, $P = 0.033$; *p*-toluidine: 979 ± 109 versus 1,304 ± 107 pg/g, $P = 0.039$). The difference for *o*-anisidine (242 ± 26 versus 328 ± 36 pg/g, $P = 0.057$) did not reach significance.

The lack of a significant effect of ETS exposure on 4-ABP Hb adduct levels is in accordance with our previous study in pregnant women (Branner et al. 1998). More recently, Grimmer et al. (2000) did not find a difference between urinary 4-ABP levels in smokers, nonsmokers and passive smokers. In the present study, an ETS-related reduction, rather than an increase, in toluidine Hb adduct levels has been observed, confirming the results of Branner et al. (1998). We still have no explanation for this paradoxical result. In smokers, these adducts are consistently elevated (Branner et al. 1998; Ronco et al. 1990) in accordance with the well-documented occurrence of toluidines in tobacco smoke (Grimmer and Schneider, 1995; Luceri et al. 1993; Patrianakos and Hoffmann 1979). However, the differences in Hb adducts from toluidines between smokers and nonsmokers were much less than those from 4-ABP. Only a small nonsignificant difference was also reported for urinary *o*-toluidine in smokers and nonsmokers (El-Bayoumy et al. 1986). It could be speculated that different dietary habits in smoking versus nonsmoking households may be responsible for the increased toluidine Hb adduct levels in children from nonsmoking homes. Dietary items themselves could be a source of these amines (Neurath et al. 1977; Vitzthum et al. 1975). Laboratory rats were found to have higher Hb adduct levels of toluidines and 4-ABP than active smokers (Green et al. 1984; Richter et al. 2000; Haussmann et al. 1998). In rats, the adducts originated most probably from the food pellets (Richter et al. 2000). Enzyme induction by high intake of vitamins (Lutz et al. 1998; Paolini et al. 1999), leading to more extensive metabolic activation of aromatic amines via *N*-hydroxylation, could be another possibility if one assumes that in nonsmoking homes healthier and more vitamin-rich diets are consumed (Subar et al. 1990; Matanoski et al. 1995). In the case of 4-ABP, a diet-related increase in Hb adduct levels may be masked by a higher ETS-related exposure to this carcinogen.

In summary, the results highlight the importance of place of residence for the exposure of children to

carcinogenic aromatic amines, and suggest that the contribution from passive smoke is comparably small.

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